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Biocatalysis to amino acid-based chiral pharmaceuticals—examples and perspectives

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Abstract

The search for and development of new pharmaceutically active structures drives the need for new enantiomerically pure compounds (EPC). Many N-containing structures can be derived beneficially from either L- or D-amino acids [K. Drauz, Chimia 51 (1997) 310–314.]. The largest growth occurs in the area of unnatural amino acids. Two examples discussed from the Degussa portfolio concern (i) D-amino acids [A.S. Bommarius, M. Kottenhahn, H. Klenk, K. Drauz, NATO ASI Series C 381 (1992) 161–174.] as components of LHRH antagonists of which the Degussa's Cetrorelix is a prime example as well as (ii) L-*tert*-leucine, occurring in a fast-growing number of pharmaceutical compounds under development [A.S. Bommarius, M. Schwarm, K. Stingl, M. Kottenhahn, K. Huthmacher, K. Drauz, Tetrahedron Asymmetry 6 (1995) 2851–2888.]. For D-amino acids, results of the hydantoinase/carbamoylase route will be presented while redox catalysis by way of reductive amination is a suitable process to L-*tert*-leucine. The number of biocatalytic applications is growing and an updated list is discussed. The presentation will also cover comparisons of biocatalysis with potentially competitive technologies such as enantioselective crystallization, chemical asymmetric synthesis, or chromatographic separation of racemates. Future trends relevant to the perspective for biocatalysis include the need for ever more complex chiral molecules as well as shortened development times in the pharmaceutical industry.

Keywords: Enantiomerically pure compounds (EPCs); Amino acids; Hydantoinase/carbamoylase system; Reductive amination

1. Introduction

Ever more often, the motivation for the investigation of biocatalytic methods and processes is the synthesis of enantiomerically pure compounds (EPCs). The responsible factors are the following: (1) There is an increasing interest in EPCs in industries other than pharmaceutical, such as the food and agro industry. (2) Diseases are now tackled with novel approaches to treatment incorporating enzymological methodology; examples are AIDS (HIV-protease inhibitors) or cancer and rheumatic inflammation (MMPI (matrix metalloprotease inhibitors)). (3) Progress has been made in the development of structure-based selection and optimization of inhibitors which are derived from structures in Nature but do not occur there.

After discussing the frame in which biocatalysis research occurs, especially in industry, two examples of the synthesis of EPCs will be presented: (i) the route to D-amino acids according to the hydantoinase/carbamoylase process

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and (ii) the synthesis of unusual L-amino acids through reductive amination. Subsequently, competitive technologies to biocatalysis are discussed. In Section 7, some future trends of the pharmaceutical intermediates industry are presented.

2. Framework for biocatalysis

There are several trends that can be observed for both biochemical and chemical processes alike [1]: (1) Catalytic reactions instead of stoichiometric ones, (2) The goal of 100% selectivity at 100% conversion, (3) High concentration of substrates, (4) Neither detrimental solvents nor frequent solvent changes, (5) The increased use of either solid or volatile acids and bases such as zeolites, ammonia or carbon dioxide as well as pH-stat techniques.

When considering to run a large-scale reaction, there are several additional points to be addressed compared to the situation of a synthesis on lab scale, among them (i) source and price of substrates, (ii) procurability and quality of the (bio)catalyst, (iii) reactor design data, (iv) downstream processing steps including the isolation of the product, and (v) the post-process treatment of analysis as well as sale and shipment to the customer.

The view on enzyme stability may serve as an example of the different perspectives between industry and academia on a problem. There are at least two ways to measure enzyme stability: (i) storage stability, often referred to as temperature stability and (ii) operational or process stability. In the former case, biocatalyst half-life ($\tau_{1/2}$) is the characteristic quantity, in the latter it is a measure of total turnover such as specific enzyme consumption (grams of enzyme preparation per kilogram product).

3. Hydantoinase / carbamoylase process

A promising route to enantiomerically pure amino acids, both L- and D-enantiomers, is based on conversion of hydantoins via hydantoinases and, additionally, carbamoylases to either D- or L-amino acids depending on the enzymes used (Fig. 1).

Hydantoinase systems to D-amino acids are more common. D-Amino acids are significant as components of antibiotics, pharmaceuticals, or

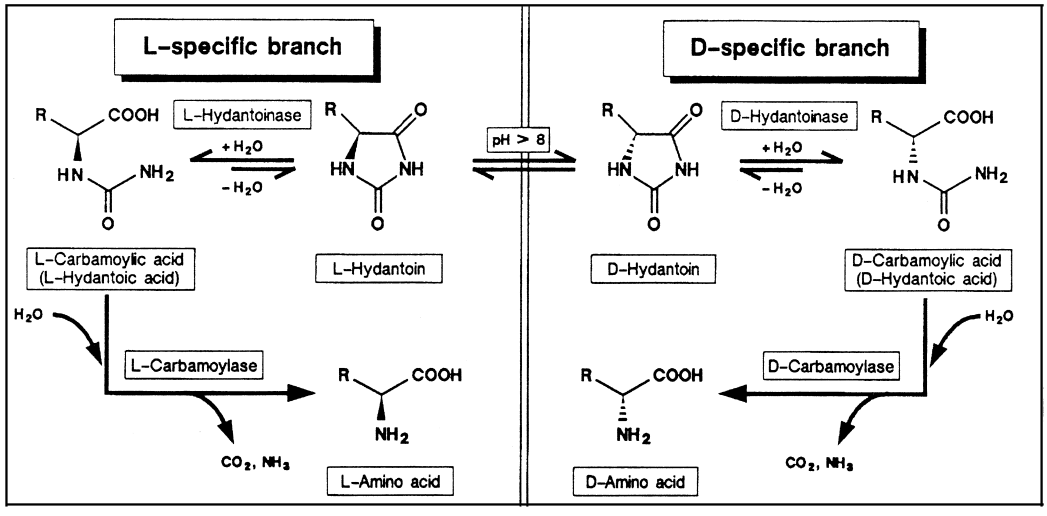


Fig. 1. Hydantoinase / carbamoylase system.

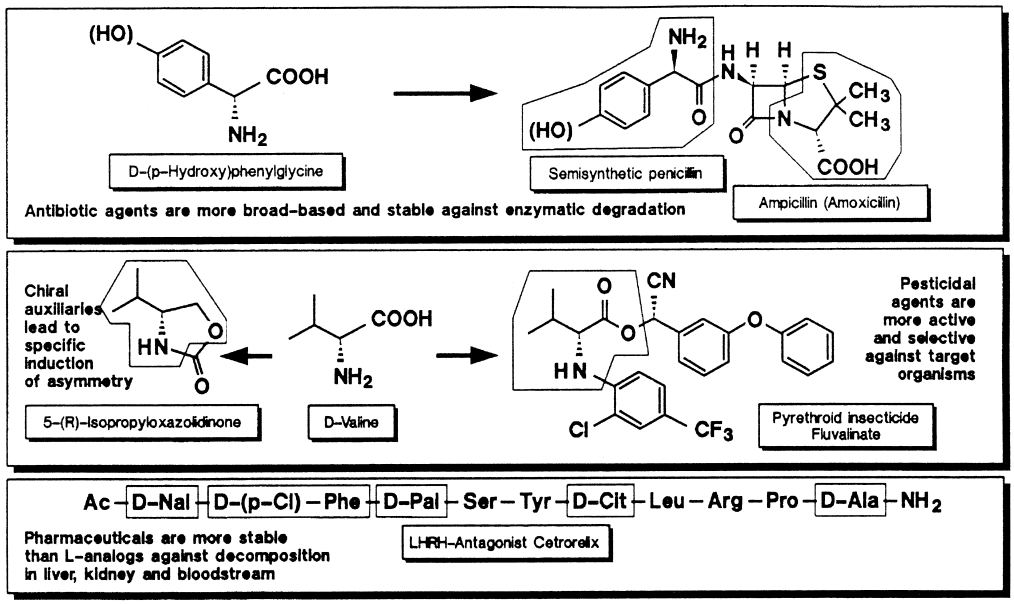


Fig. 2. Significance of D-amino acids.

pesticides that are often more active but also more stable that the L-containing analogs (Fig. 2).

In nature, D-hydantoinases do not convert hydantoins but often the six-membered analogs, dihydropyrimidines, thus most hydantoinases are D-dihydropyrimidinases rather than D-hydantoinases. Above about pH 8, 5-mono-substituted hydantoins racemize owing to their acidic hydrogen through keto-enol-tautomerism, however, the rate constant of racemization strongly depends on the residue in 5-position

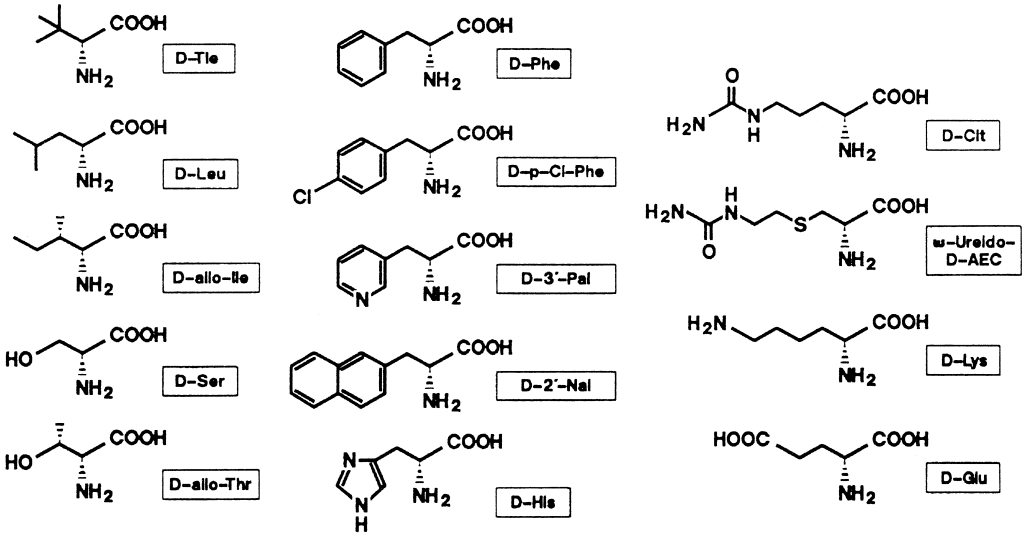


Fig. 3. D-amino acids produced on pilot- and full-scale.

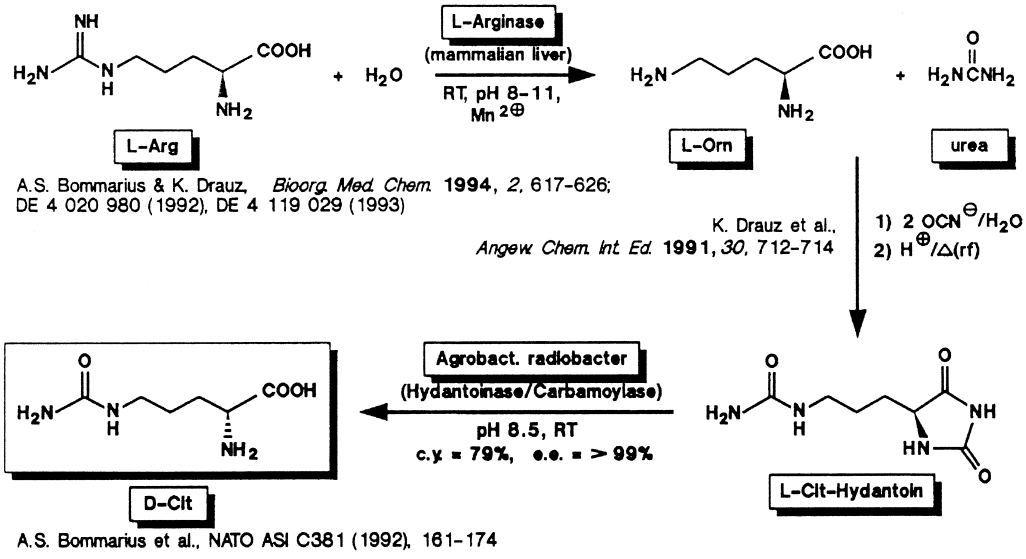


Fig. 4. Synthesis route to D-citrulline.

($\tau_{1/2}$: phenylhydantoin 0.3 h, benzylhydantoin 5 h and isopropylhydantoin 56 h) [2].

Fig. 3 is a non-exhaustive list of D-amino acids produced in pilot and bulk quantity by Degussa using different hydantoin biocatalyst systems. From the figure, the broad range of substrate specificity and thus the versatility of the biocatalyst system is apparent.

A particularly suitable example for illustration of the versatility of the chemoenzymatic approach to rare amino acids is the route to D-citrulline (D-Cit). Starting from L-arginine (L-Arg), enzymatic hydrolysis with arginase to L-ornithine (L-Orn) [3–6] is followed by double cyanation with two equivalents of cyanate to *N*-α-carbamoyl-L-citrulline [7] which is cyclized under acidic conditions to γ-ureidopropyl-hydantoin (Cit-hydantoin), the substrate for the D-hydantoinase/D-carbamoylase system [8]. The chemical yield is 79%, e.e. is $>99\%$ (Fig. 4).

4. Reductive amination

Enantiomerically very pure L-amino acids can be obtained elegantly and efficiently by reductive amination of α-keto acids by amino acid dehydrogenases. Fig. 5 features the case of leucine dehydrogenase (LeuDH) which converts hydrophobic α-keto acids to the respective L-amino acids such as L-*tert*-leucine, L-neopentylglycine or analogs with the help of the cofactor NADH. The resulting form of the cofactor, NAD^+, is regenerated to NADH by the well-known reaction employing FDH and ammonium formate. The formate reaction is irreversible and the co-product CO_2 can be easily separated. This regeneration method, brought to scale by Degussa in cooperation with professors Kula and Wandrey [9], is now considered the method of choice for running reductive amination reactions [10].

Reductive amination is a useful route to a whole series of products with GluDH, PheDH and LeuDH. ^{15}N can be incorporated easily as $^{15}NH_3$ into labelled amino acids such as ^{15}N-glutamate with GluDH. PheDH is used to generate L-homophenylalanine, a component of ACE inhibitors [11]. Branched-chain amino acids such as L-*tert*-leucine can be used for templates in asymmetric synthesis and as building blocks for pharmaceutically active compounds. L-Tle itself is a component of several

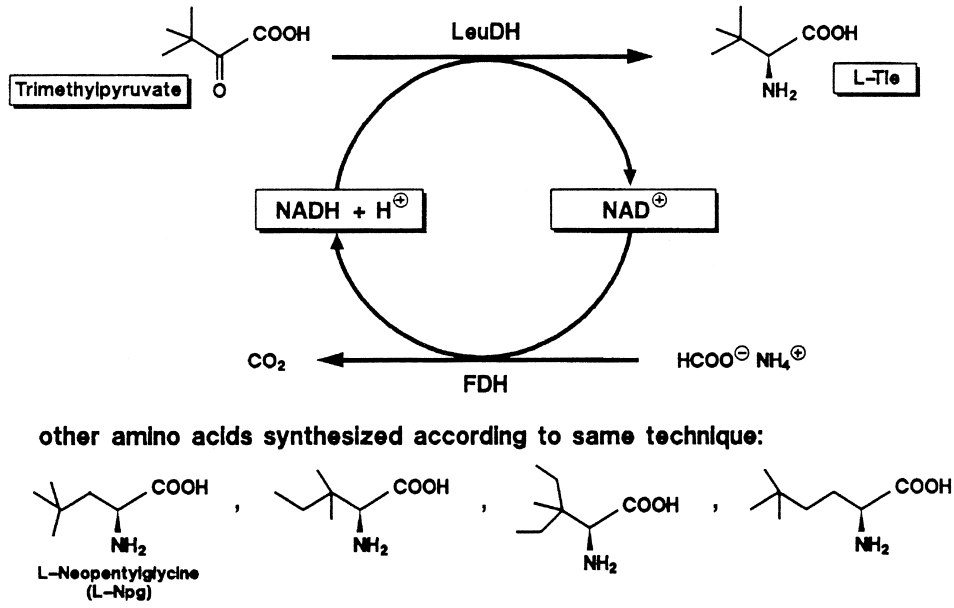


Fig. 5. Synthesis of L-*tert*-leucine and analogs via reductive amination.

pharmaceutical development projects as tumor-fighting agents or HIV protease inhibitors against several diseases such as different tumors, rheumatic arthritis and AIDS (Fig. 6). Also, a whole range of ligands for asymmetric catalysts has been developed with L-Tle, mostly based on the oxazolidine moiety.

The substrate specificity of different LeuDHs has been investigated and compared [12]. The resulting reaction rates have been attempted to

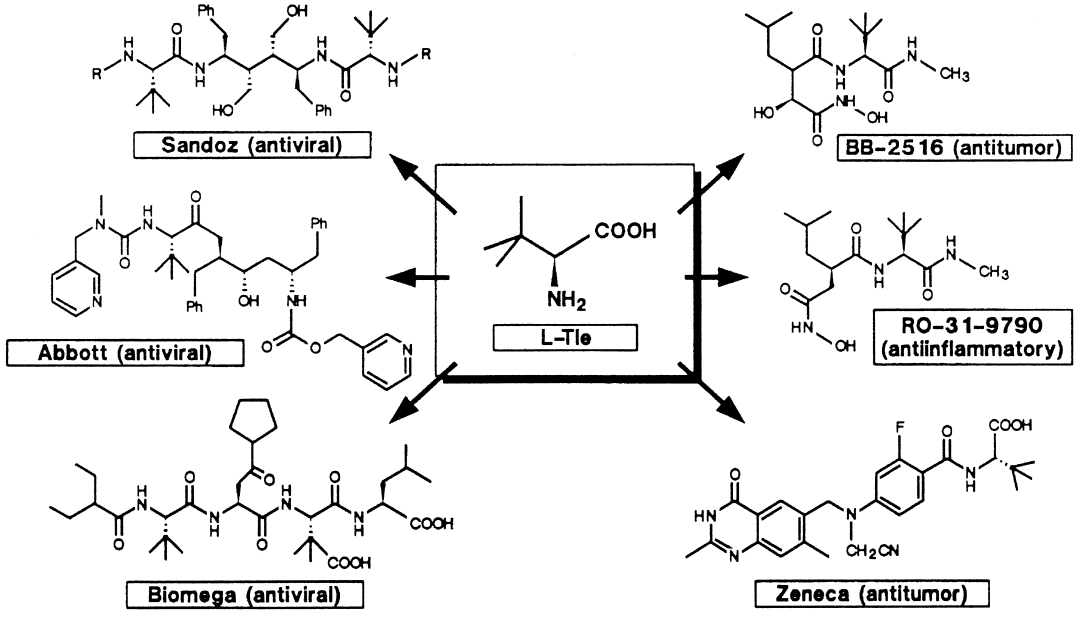


Fig. 6. L-*tert*-Leucine as component of pharmaceutically active structures.

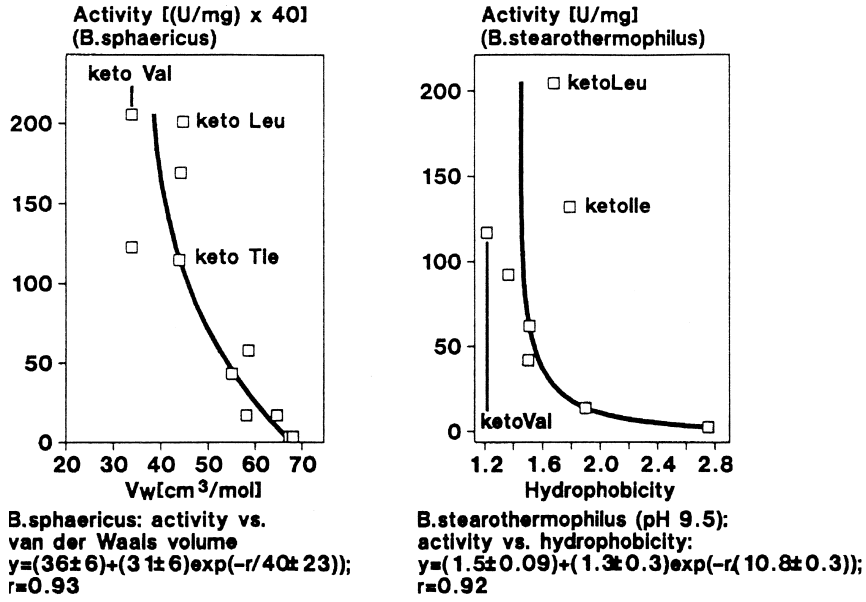


Fig. 7. Substrate specificity of LeuDH.

be correlated with structural parameters of the substrate sidechains such as hydrophobicity or van der Waals radius (Fig. 7).

However, the studies of structure–activity relationships have not been very revealing: the correlations are moderate and do not reveal much beyond the influence of hydrophobicity or the van der Waals radius. Comparison of the substrate specificity of LeuDH from different sources reveals a striking similarity among the different LeuDHs. At pH 8.0, 2-oxo-3-methylbutyric acid (keto-valine) is the most active

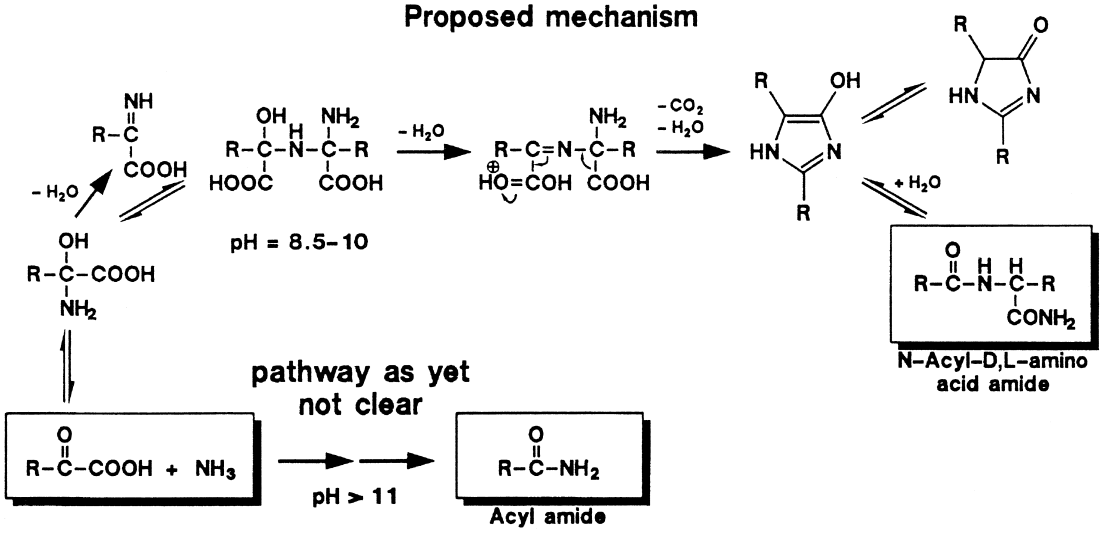


Fig. 8. Reactivity of keto acids with ammonia.

substrate among the common aliphatic keto acids.

The limitations of the process are not set by enzymatic but by chemical reactions: at high concentrations of ammonia and keto acids chemical formation of by-products in the very reactive solution occurs. The nature of the by-products has been identified and strong evidence exists for the mechanism leading to the formation of these by-products, *N*-acyl-D,L-amino acid amides; they form according to the upper pathway in Fig. 8.

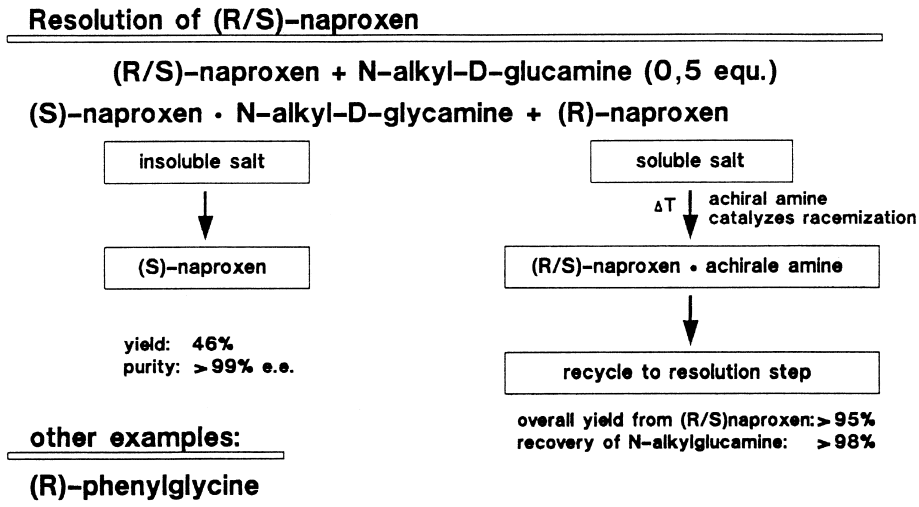
The system has been scaled to the large pilot scale and, in some cases, hundreds of kilograms of amino acids have been produced by this route. Even with slow substrates, this process can be scaled up. As an example, 30 kg of L-neopentylglycine have been obtained after four days reaction time at 40°C and pH 9.0 (20 kU LeuDH, 40 kU FDH); while this constituted only 74% of the theoretical yield, another 4.3 kg (13% of theoretical yield) were recovered as the insoluble by-product α-*N*-neopentyl-neopentylglycinamide.

5. List of scaled-up biocatalytical processes

By now, plenty of biotransformations have been developed to industrial scale; leading is the glucose–isomerase process ($> 10^6$ t/a) followed by production of cocoa butter and acrylamide and leading down to smaller productions in the lower ton range, most of them intermediates to pharmaceutically active compounds such as (*R*)-glycidyl butyrate, prepared with lipases, or to food and cosmetics compounds such as

Table 1
Industrial-scale biotransformations

Scale, enzyme	Product	Reactor	Company
> 1,000,000 t / a			
Glucose isomerase	fructose	fixed-bed, IME	various
> 10,000 t / a			
Nitrile hydratase	acrylamide	whole cells	Nitto
Lipase (mucor miehei)	cacao butter	fixed-bed, IME	Fuji Oil, Unilever
> 1000 t / a			
Penicillin amidase	6-APA	fixed-bed, IME	various
Aspartase	L-Asp	fixed-bed, IME	Tanabe
Thermolysin	aspartame	soluble enzyme	Tosoh/DSM
Hydantoinase	D-Phg	resting cells	Kanegafuchi
Hydantoinase/carbamoylase	D-*p*-OH-Phg	resting cells	Recordati
Aldonolactonase	D-pantothenic acid		Fuji Pharmaceuticals
> 100 t / a			
Fumarase	L-malic acid	fixed-bed, IME	Tanabe
Aminoacylase	L-Met, L-Val	EMR	Degussa
Aminoacylase	L-Met, L-Phe, L-Val	fixed-bed, IME	Tanabe
β-tyrosinase	L-Dopa		Ajinomoto
Lipase (*Pseudomonas*)	(*S*)-3-acetylthioisobutyrate	IME, EMR	DSM-Andeno, Tanabe
Hydroxylase	L-carnitine	whole cells	Lonza
> 10 t / a			
Lipase	(*R*)-glycidylbutyrate	batch	BASF, DSM
Trans-glucosidase/lipase	α-butylglucoside ester	fixed-bed, IME	BioEurope/Solabia
Dextransucrase	gluco-oligosaccharides	fixed-bed, IME	BioEurope/BioEcolia



Scheme 1. Resolution of *R*/*S*-naproxen.

oligosaccharides, made with dextransucrase (Table 1).

6. Competitive technologies

Despite the upward trend for biocatalysis in the field of EPCs other competitive technologies in many cases often demonstrate similar performance:

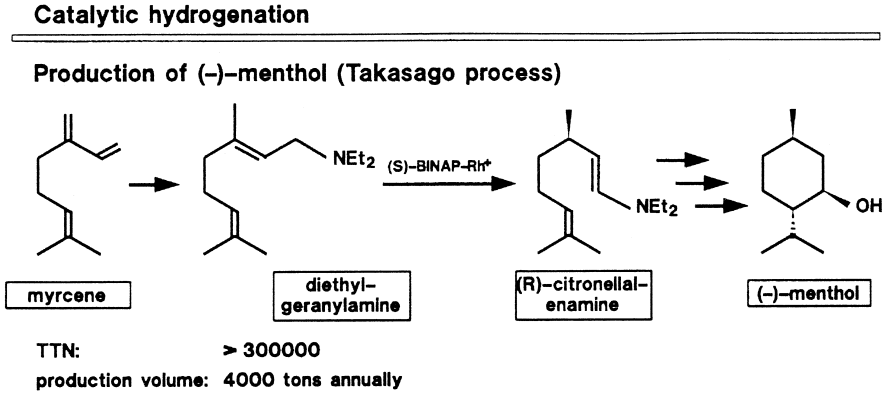
(i) Enantioselective crystallization is the method of choice for the production of (*S*)-naproxen and (*R*)-phenylglycine (Scheme 1).

(ii) Asymmetric synthesis can be successfully employed for large-scale processes in selected cases, especially in catalytic hydrogenation (Scheme 2).

(iii) Chromatographic separation of racemates will be increasingly common. At Degussa, the racemate of D,L-*tert*-leucine has been separated on the molar scale.

7. Future trends in the markets for pharmaceutically active compounds

In the near future, three important trends can be discerned for the area of pharmaceuticals and



Scheme 2. Catalytic hydrogenation.

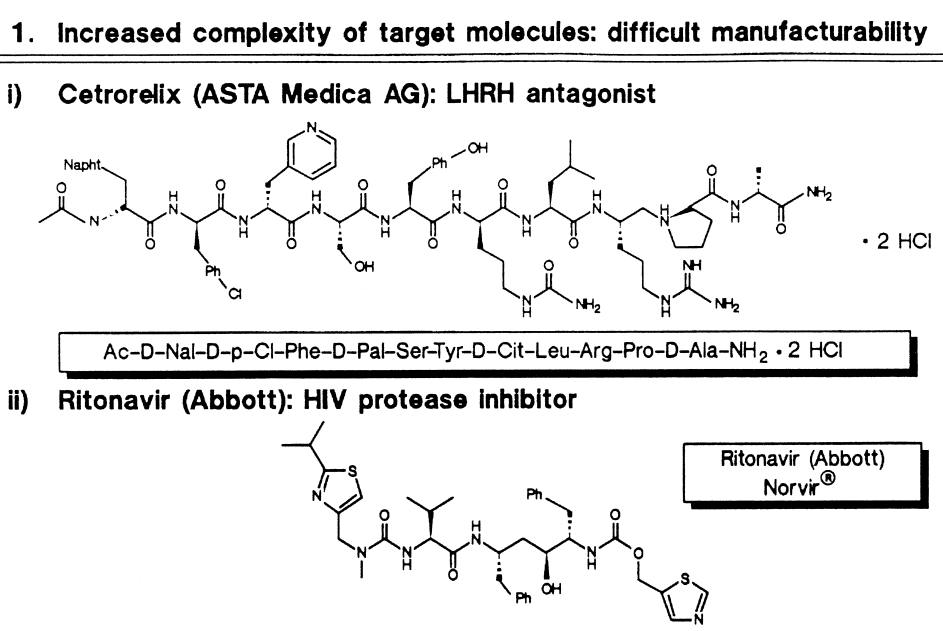


Fig. 9. Complexity of target molecules.

the field of process development for pharmaceutical intermediates.

(1) The synthesis routes to pharmaceutically active molecules become more and more complicated. This raises the questions whether such molecules, usually featuring several chiral centers, can be synthesized at all within given process and cost constraints (Fig. 9).

(2) Owing to the high costs of development of novel pharmaceuticals, the industry seeks to cut the development times in half. This would increase the time span for utilizing patent pro-

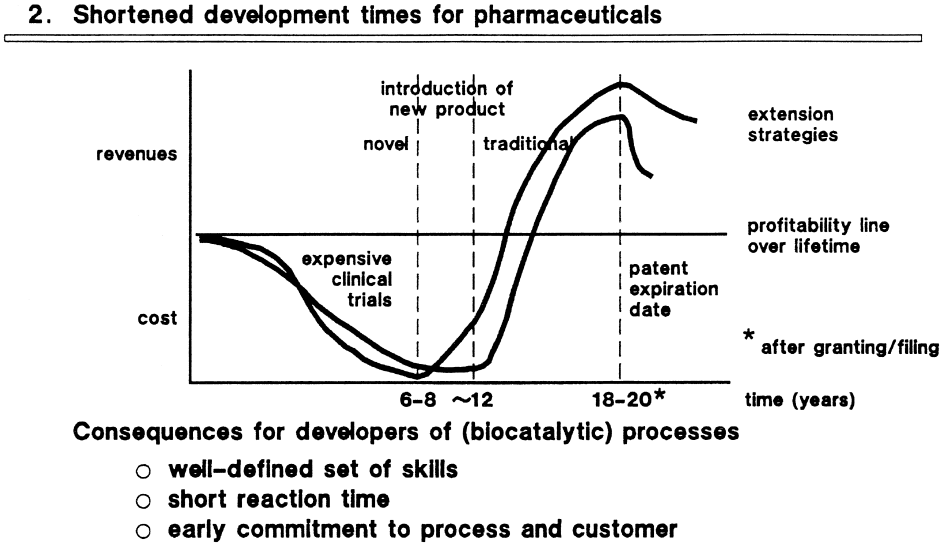


Fig. 10. Shortened development times.

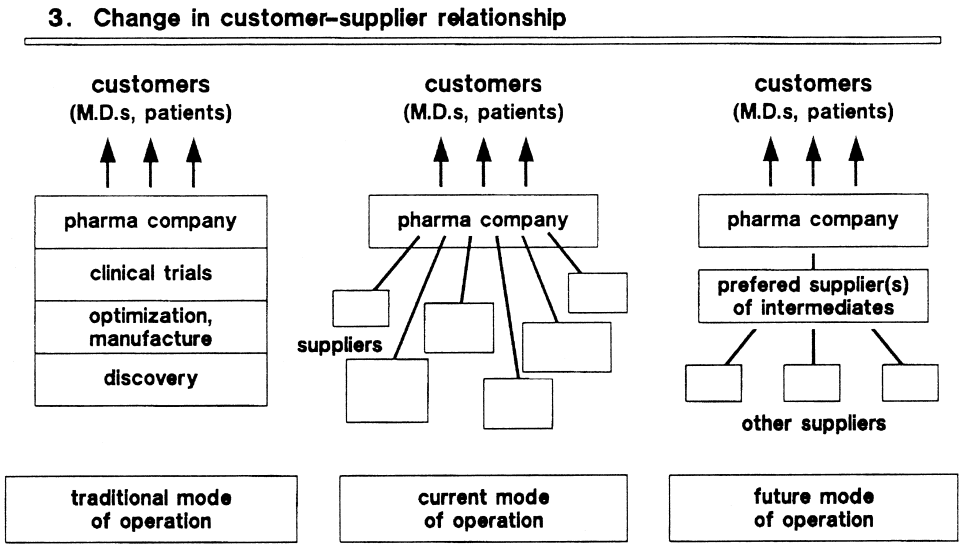


Fig. 11. Customer–supplier relationship.

tection between market introduction of a new compound and expiration of patent protection (Fig. 10).

(3) The customer–supplier relationship in the industry will change: While traditionally pharmaceutical companies have kept in-house the whole development and production chain from discovery to marketing, production will be outsourced to dedicated fine chemicals producers in the future. Moreover, the technological capabilities of small specialized suppliers will likely be bundled by larger 'systems integrators', companies that deliver larger portions of a compound all the way to the final bulk active (Fig. 11).

In conclusion, the following developments should be summarized: (1) Biocatalysis is an expanding field both scientifically and applications-wise, (2) Competitive technologies such as asymmetric catalysis catch up rapidly, (3) New challenges appear on the technology front: enhanced requirements with respect to selectivity and stability of catalysts, more complicated target molecules and increased cross-disciplinary work teams, (4) New challenges also appear on the market horizon: more intense competition, higher costs for product development coupled with simultaneously decreasing development times.

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